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Introduction. W81XWH-04-1-0204 HOXC Family Gene Expression in Prostate Cancer: a Mechanism Contributing to Androgen Independence

This was a Hypothesis Exploration award to explore the idea that overexpression of genes of the HOXC cluster that we observed in earlier studies inhibits androgen-mediated gene induction and may therefore predispose prostate cancer cells to become androgen-independent for growth. Experiments were proposed to test these predictions.

Main Body: W81XWH-04-1-0204 HOXC Family Gene Expression in Prostate Cancer: a Mechanism Contributing to Androgen Independence

This application proposed that HOXC gene overexpression would inhibit androgen-mediated gene expression. More speculatively, it proposed further that this inhibition may predispose prostate cancer cells to become partially androgen-independent for growth even before the imposition of androgen ablation therapy. A series of experiments in cell culture models and in cell-free transcription were proposed to address these two hypotheses. Experimental data described below support the contention that androgen-mediated transcription is inhibited by HOX overexpression. Curiously, the activity of the vitamin D receptor, another nuclear receptor that plays a key role in the control of prostate cancer growth, can be either inhibited or potentiated by HOXC overexpression. The data suggest that the role of HOXC overexpression on prostate cancer may be a complex one. Addressing the second hypothesis that HOXC overexpression could affect the development of androgen-independence is experimentally tricky to design in a way to adequately and appropriately test the hypothesis. To lay the groundwork for such experiments a more thorough characterization of the effects of androgens on prostate cancer lines, especially LNCaP has led to and expanded examination of the mechanisms underlying the biphasic actions of androgens on prostate cells and the complex interplay of the controls governing cell cycle and apoptosis. Specific experimental findings are described in the following section.

Key Accomplishments: W81XWH-04-1-0204 HOXC Family Gene Expression in Prostate Cancer: a Mechanism Contributing to Androgen Independence

Task 1a. Complete. Probasin luciferase vectors were obtained from Dr. Robert Matusik and used as an androgen-dependent reporter in these studies along with a mouse mammary tumor virus luciferase reporter.

Task 1b. Complete. We have shown that HOXC6, HOXC8, and HOXB13 all progressively inhibit androgen receptor-mediated induction of two androgen-responsive promoters, the probasin (Figure 1) and mouse mammary tumor virus promoters in LNCaP cells. This inhibition cannot be accounted for by effects of HOX genes on the expression of androgen receptors (Figure 2). Beyond what was proposed, we have shown that, in addition to the androgen receptor, HOXC overexpression inhibits the activity of estrogen, progesterone, and glucocorticoid receptors and that the effects of HOXC overexpression is not confined to prostate-derived cells or to epithelial cells.

Task 1c. Complete. We have obtained two luciferase vectors with vitamin D responsive promoters including the 24-hydroxylase promoter (*c.f.* Figure 3).

Task 1d. Complete. The vectors have been used in transfection assays to assess the functional interaction of the vitamin D receptor and HOXC genes. Remarkably, while HOXC6 and HOXC8 overexpression inhibit the vitamin D receptor-mediated gene induction in ALVA31 prostate cancer cell lines, it potentiates vitamin D receptor action in LNCaP cells (Figure3).

Task 2a. Complete. Expression vectors for HOXC8, HOXC6, as well as other HOX genes were constructed. A decision was made to express HOX genes as GST-tagged versions rather than as FLAG-tagged proteins using baculovirus vectors for ease of expression and purification.

Task 2b. Partially complete. Androgen receptor has been expressed using baculovirus vectors and purified.

Task 2c. Not complete. The androgen receptor has not exhibited activity in the in vitro transcription system in initial experiments.

Task 2d. Not complete. This task relies on the successful completion of task 2c before it can be attempted in a meaningful way.

Task 2e. Partially complete. Initial chromatin immunoprecipitation experiments have shown that HOXC8 overexpression abrogates androgen-induced histone acetylation at a transiently transfected androgen-responsive promoter. We have not yet shown whether this inhibition is accompanied by a decreased loading of the histone acetyl transferase coactivators, CBP or p300.

Task 3a. Partially complete. Plasmid expression vectors for HOXC8 have been constructed but not retroviral vectors as yet.

Task 3b. Partially complete. We have characterized the androgen-dependence of NRP-152 cells and have not been able to repeat the published claims that this cell line is androgen-dependent for growth in culture. We have been unable to characterize the EPN cell line because, despite repeated assurances from the Italian group who developed this line, they have not sent the line to us.

Task 3c. It was proposed to begin the development of cell lines overexpressing HOX genes to assess the impact on growth in androgen-driven and androgen-deficient conditions. In order to understand the impact of HOX overexpression on androgen signaling in prostate cancer cell models, we have spent a great deal of effort more thoroughly characterizing how androgens effect the growth of the widely-used prostate cancer cell model, LNCaP. We have show that androgens have a biphasic action on these cells. At low doses, ~10-30 pM, the synthetic androgen R1881 stimulates growth but at higher doses, ~10 nM, it inhibits growth and stimulates apoptosis of these cells (Figure 4). This may have clinical ramifications as it implies that incomplete androgen blockade may stimulate rather than inhibit the growth of prostate cancer. Experiments are ongoing to further characterize the biphasic actions of androgens but recent experiments indicate that we have been able to select LNCaP cell variants that are androgen-independent for growth but still retain the apoptotic response and others that are androgen-dependent for growth but do not undergo growth arrest and apoptosis at higher doses of androgen. These variants could prove to be valuable tools in elucidating the complex role of androgens in the prostate and prostate cancer.

Reportable outcomes: W81XWH-04-1-0204 HOXC Family Gene Expression in Prostate Cancer: a Mechanism Contributing to Androgen Independence.

These studies were presented at the 87th Annual Meeting of the Endocrine Society. Abstract P2-663. A copy of the abstract has been appended.

A grant application on the role of HOX in predisposing prostate cancer cells to androgen-independence that relied on data generated from these studies was submitted to NIH. Unfortunately, the application did not receive a fundable priority score.

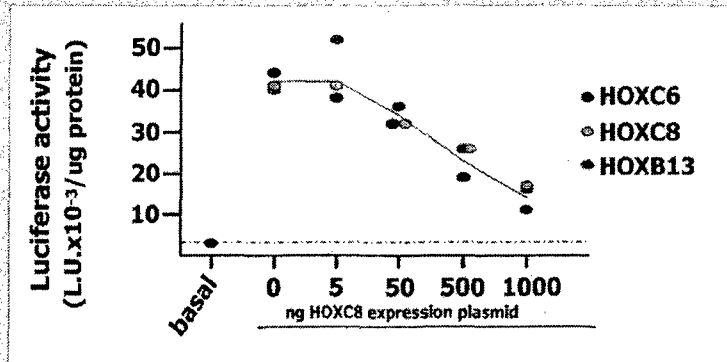
A graduate student, Sunshine Daddario, has elected to pursue this project for her thesis studies. She submitted a pre-doctoral training grant application to the 2005 Congressionally Directed Medical Research Program on Prostate Cancer and her application has been approved for funding.

Vectors have been constructed for the bacterial expression of various HOX genes as GST-tagged versions for facile protein purification. Mammalian expression vectors also have been constructed for several of the HOX genes.

LNCaP variant cell lines described above have been developed and are undergoing further characterization.

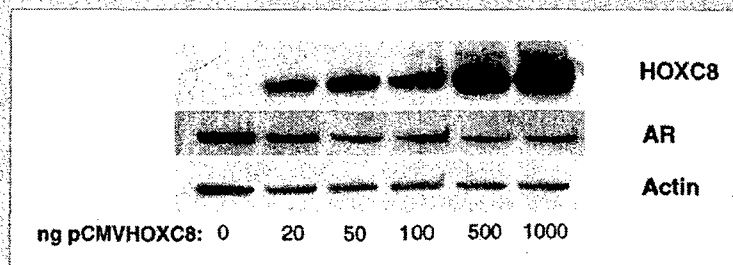
Conclusions: Overexpression of HOXC genes inhibit transcriptional induction mediated by the androgen receptor and other steroid receptors as well. Interestingly, activity of the vitamin D receptor, also a member of the large nuclear receptor family, can be either inhibited or potentiated depending on the cell type. The impact of inhibiting androgen action in prostate cancer may be complex and further studies are required to ascertain whether HOXC gene expression, by limiting the growth stimulatory actions of androgens, may predispose prostate cancer cells to become androgen-independent for growth.

Figure 1



Increased HOXC6, HOXC8, or HOXB13 expression inhibits androgen induction of the probasin promoter. LNCaP cells were transfected with a probasin-luciferase reporter vector and increasing amounts of a HOXC8 expression vector. (Total transfected plasmid was held constant by balancing with empty expression vector). Cells were treated with 10 nM R1881 for 24 hours.

Figure 2

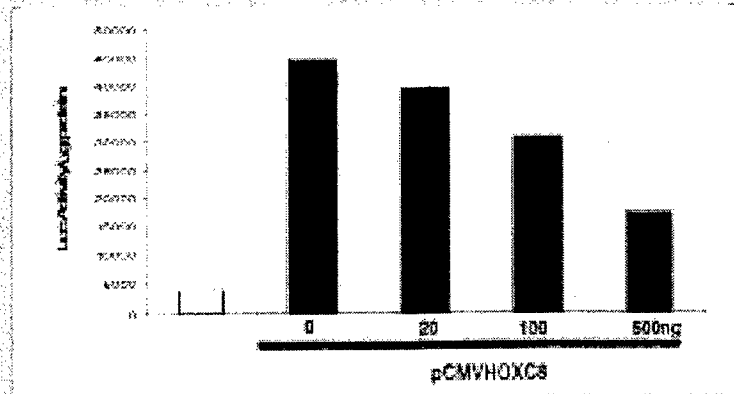


The inhibition of androgen action by HOXC8 expression is not due to an inhibition of AR expression. Western blot analysis of transfection experiments show that increasing expression of HOXC 8 by transfecting increasing amounts of HOXC8 expression plasmid does not impact expression of androgen receptor. Thus, HOX genes inhibit androgen mediated gene induction by a mechanism other than simply suppressing AR expression.

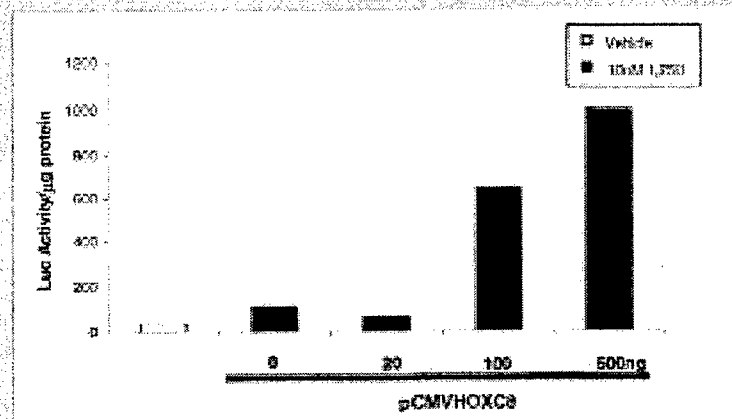
Figure 3

Differential Effects of HOXC8 on Vitamin D Receptor Signaling in Human PCa Cells

A) ALVA-31



B) LNCaP



Increased HOXC8 expression exhibits differential effects on vitamin D receptor-mediated signaling in human PCa cell lines. Reporter assays as described in Figure 1. (A) Increased HOXC8 expression inhibits vitamin D induction of the 24-OH vitamin D responsive promoter in ALVA-31 PCa cells; (B) Increased HOXC8 expression potentiates vitamin D induction of the 24-OH vitamin D responsive promoter in LNCaP PCa cells.

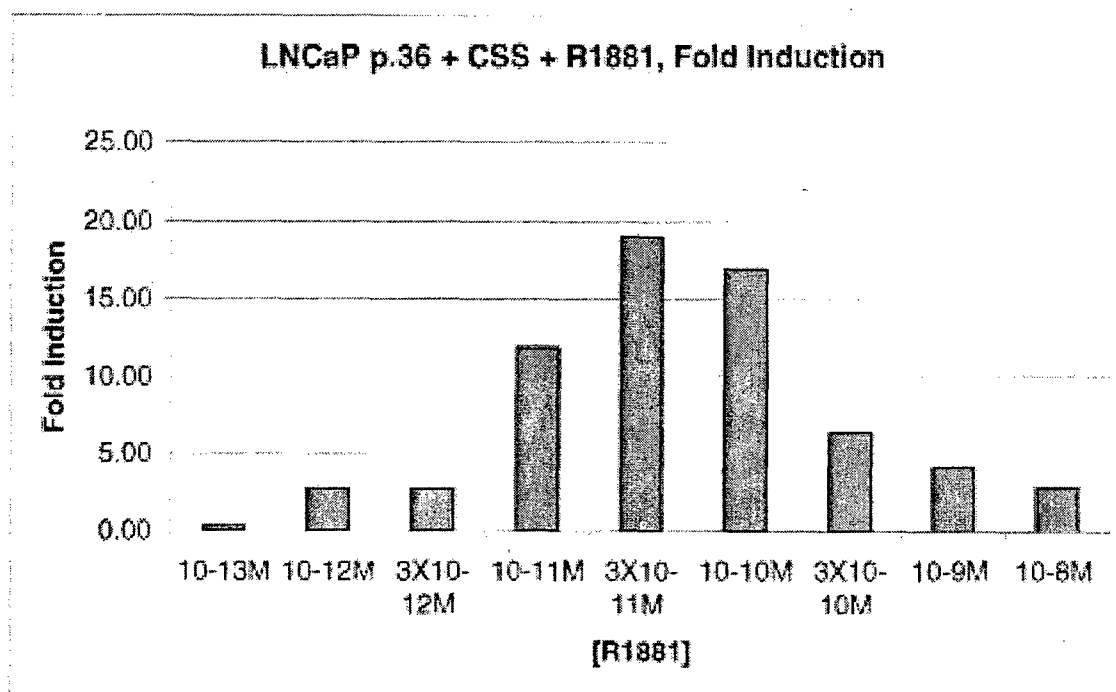


Figure 4. Induction of net growth in LNCaP prostate cancer cells grown in medium supplemented with charcoal-stripped serum plus the indicated dose of the synthetic androgen R1881. DNA content was measured after 6 days of treatment. Fold induction is relative to cells not treated with R1881.